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UNINUCLEATED AECIDIOSPORES IN CAEOMA NITENS, AND ASSOCIATED PHENOMENA [1]

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INTRODUCTION

The cytology of the mallow rust *Puccinia malvacearum* has been studied by Werth and Ludwigs (*15*), Moreau (*12*), and others.[2] Nothing was discovered that suggested to these investigators that the omission of the spermogonial structures has any special significance whatever in the life of the rust. The discussions and controversies in regard to this short-cycled Puccinia have largely centered around questions as to time and method of infection and of over-wintering. Cell fusions take place in the sorus, each binucleated cell of the teleutospore becomes uninucleated by nuclear fusion, divisions occur and a 4-spored promycelium is developed.

In describing the spore forms of species of rusts mycologists frequently state that spermogonia (pycnia) are unknown or wanting. A preliminary study of the short-cycled orange-rust, *Caeoma nitens*, on blackberries, has led the writer to suspect that the suppression of spermogonia in the life of this rust may follow as the result of something more fundamental than that which leads to the omission of the pycnidial stage by some species of the ascomycetes. In other words, if the facts can be interpreted in the light of phylogenetic developments, it may be discovered that the omission or suppression of the spermogonial stage is in reality of sexual significance although the spermatia in themselves take no part in a process of fertilization. A brief résumé of the occurrences which led the writer to give closer attention to the cytological details in the growth of the orange-rust of Rubus may perhaps serve as a statement of the main features presented in this paper.

Several years ago while growing rusts in the greenhouse for class use at Columbia University, the writer observed that in certain cases of infection of blackberry by the short-cycled orange-rust, no spermogonia were developed on plants whose leaves were maturing the aecidial stage. This was in striking contrast with what is commonly known to occur. One of the most familiar sights in this country is the appearance of etiolated blackberry shoots whose leaves are covered with spermogonia when the infected sprouts emerge from the ground some days in advance of the others. In reporting on the results of work relating to infection of blackberries with the "haploid" generation of the orange-rusts (*3*), it was pointed out that as the inoculated plants of the Iceberg variety began to show signs of infection, no spermogonia could be found, even though the shoots were several inches high and the young aecidia were beginning to develop. That this omission of the spermogonial stage was not due specifically to peculiarities of this host, which is

[1] Received for publication June 30, 1924—issued Nov., 1924.

[2] Reference is made by number (italic) to "Literature cited," p. 1058.

an albino variety, was evident later when plants of the Mereereau variety similarly infected also failed to show spermogonia. Infected plants of both varieties were observed again the following year to see if spermogonia might not develop the second year after infection. At no time in the past three years has the rust on these plants produced spermogonia. This fact was the more interesting because on those plants of the Ancient Briton and Eldorado varieties which had been infected with the short-cycled rust in one of the experiments, spermogonia had always preceded the aecidia. The rust on a Taylor blackberry which was infected when received from the nursery, developed both spermogonia and aecidia, but the rust on other plants of this variety which later were artificially infected did not develop spermogonia.

During the spring of 1923, two root shoots of the Iceberg variety were inoculated with orange-rust, the one was infected by sowing sporidia from aecidiospores of the strain from the Ancient Briton which had developed spermogonia, the other Iceberg was infected with spores from rust on wild blackberry. Both plants inoculated became systemically infected and developed aecidia in 1924. The first plant showed many spermogonia on the leaves from the basal shoots as soon as they developed; the infected basal shoots from the other plant developed only aecidia, no spermogonia. While endeavoring to account for the occasional suppression of the spermogonial stage of the short-cycled orange-rust, it was discovered that aecidiospores of those strains of the rust omitting the spermogonial stage are uninucleated. Cell fusions do not regularly take place in the aecidium primordium. The uninucleated spores develop promycelia when they germinate but the promycelia are 2-celled, and each cell produces only one sporidium.

As the figures to be discussed later show, the number of cells in a promycelium is not constant for any strain. Promycelia are reported as 2-celled, or 4-celled, when one sees large numbers that are 2-celled or 4-celled, as the case may be. If no spermogonia could be distinguished with a hand lens on an infected leaf it would be reported as without spermogonia, when a more careful examination with a higher-powered microscope might prove that poorly developed or vestigial spermogonia were present. In the discussion which follows, the statement as to absence of spermogonia should be read with this understanding.

The absence of spermogonia may seem to be a point particularly stressed in the following account, when it is clear that the facts regarding the production of 2-celled promycelia which function is the feature which is being set forth as a new departure from the regular order of promycelia in the rusts. It will be shown later, however, that both of these features are merely the outward expression of something of relatively far greater significance fundamentally, and that is, that an aecidium of the orange-rust of Rubus may arise without cell fusions and that the spores matured without such cell fusions are generally uninucleated.

Since the long-cycled, as well as the short-cycled orange-rusts, have both been proved to be widely distributed in America on a number of species of Rubus, further evidence on the mere question of their distribution in the form of extensive tables showing germination tests is unnecessary, except as some new element enters the discussion. The discovery of uninucleated strains with 2-celled promycelia makes it highly desirable that further germination tests be made in such a way as to record the different types of promycelia as correlated with the relative abundance of spermogonia, as well as the size, form, and color of the spores. The abundance of the strain which develops 2-celled promycelia may be judged from the following accounts of collections gathered at random in various localities in one season.

OCCURRENCE OF CAEOMA NITENS WITHOUT SPERMOGONIA

Miss Ruth Colvin, who was assisting the writer with this work, made one collection of orange-rust about every mile from wild blackberries May 24 along the road between Washington and Fairfax, Va. The results of her germination tests show that only 11 of the 30 collections were of the strain whose spores develop 2-celled promycelia; only one long-cycled specimen was obtained, in this case the host happened to be the black raspberry, *Rubus occidentalis.* In most regions around Washington one would certainly find on such a trip blackberries infected with the long-cycled form.

Miscellaneous gatherings on wild blackberries near Upper Marlboro, Md., always contain some specimens which are long-cycled. Twenty-two specimens were gathered from wild blackberries in this region June 2. The presence or absence of spermogonia was determined in the field by Prof. R. A. Harper and Dr. C. L. Shear. Of the 22 gatherings, seven happened to be long-cycled; the spores from the eight specimens without spermogonia produced 2-celled promycelia, and those from three gatherings developed only 4-celled promycelia. The rust on three different plants was of the type which develops spores of two sorts; some produce long germ tubes characteristic of the long-cycled forms, others germinate with 4-celled promycelia. The specimens which were proved to be of the long-cycled type were also characterized by the greater abundance of spermogonia.

The results of germination test of specimens collected May 9 at Chadbourn, N. C., and May 10 to 12 at Louisville and Vidalia, Ga., are such as to suggest that the strain which does not develop spermogonia normally and whose spores form 2-celled promycelia, greatly predominates in the South. The specimens tested, however, were gathered within a limited area in each place, so that a further survey would be necessary in order to determine this point. Of the 18 specimens tested only one was found which developed 4-celled promycelia. This was found on *Rubus trivialis* at Louisville, Ga.

Miss Colvin sent in 11 specimens gathered May 30, between Washington, D. C., and Gettysburg, Pa. No spermogonia could be found on leaves of six specimens whose spores developed 2-celled promycelia. Spermogonia were abundant on four different specimens, three of which were short-cycled and formed 4-celled promycelia. The other one was long-cycled. One specimen showed a few spermogonia and some 4-celled promycelia were found in the culture but 2-celled promycelia were much more abundant. A few other cases of infection found elsewhere have proved to be of a type where 2-celled promycelia are developed, although spermogonia are present on the leaves.

On June 3, 19 collections were obtained at the experimental farm at Bell, Md. Leaves on nine infected plants showed spermogonia and the rust was of the 4-celled promycelium type in six cases; the other three gatherings were long-cycled. Spores from nine specimens showing no spermogonia developed 2-celled promycelia. One large plant with a number of canes was clearly infected with two different strains of orange-rust. Leaves on most of the canes showed an abundance of spermogonia. Here the rust was long-cycled. No spermogonia could be found on leaves on one cane and here the rust was short-cycled, the promycelia being 2-celled (see Pl. 1, B, D).

On June 12, Professor Harper and Dr. F. D. Fromme sent in nine specimens from rusted blackberries gathered on the road to Mountain Lake, Va. No spermogonia could be found on the three specimens whose spores germinated with 2-celled promycelia. Five specimens showed an abundance of spermogonia. Only one of this number proved to be short-cycled, 4-celled promycelia being formed as the spores germinated.

Dr. N. E. Stevens, while en route by automobile from Washington, D. C., to Providence, R. I., June 4 to 6, sent in 35 specimens of orange-rust on blackberries and dewberries. The germination tests show that the strain which develops spermogonia and 4-celled promycelia was more abundant along this route at that time than was the strain without spermogonia and producing 2-celled promycelia; only 7 specimens were of the latter type, while 23 were of the type which develops 4-celled promycelia.

Whether the strain which develops 2-spored promycelia may not be relatively more abundant as one goes farther South can not be said with certainty. When the result of germination tests of rust collected from northern New Jersey and central New York are compared with those from North Carolina and Georgia, it might seem that the strain without spermogonia which develops 2-celled promycelia is more abundant in the South. All of the specimens collected at Junius, Geneva, and Albany, N. Y., June 16 to 18, showed at least some spermogonia. Only a few localities were visited but it is possible that the strain in which the spermogonial structures are poorly developed or omitted is not so common in the North. Of the 24 specimens tested 5 were long-cycled. Two-celled promycelia developed from spores from 2 specimens.

Several other collections were made at Ridgewood, N. J. Owing to the excessively hot weather prevailing and the delay in the mails, very poor germination was obtained. All of these specimens collected June 19 on wild dewberry showed spermogonia when carefully examined later. When germination was such as to enable one to determine the nature of the promycelium, it was found to be 4-celled. A few long germ tubes were seen in cultures from 6 specimens on blackberry. Very likely this rust was long-cycled and further search later will result in the discovery of telia in this vicinity.

All of the leaves in specimens collected at Wells Beach, Me., June 22, on wild dewberry and blackberry, were covered with spermogonia. Tests proved that the rust here is long-cycled. Dr. D. Folsom has sent in specimens of orange-rust collected in Maine. Germination was tested at Orono and the rust found to be short-cycled.

A number of plants of different varieties and species of Rubus infected with orange-rust are grown in the greenhouse at Arlington, Va., from year to year. In every case here so far tested, where spermogonia are readily seen with a hand lens, the rust is either long-cycled, or if short-cycled, it develops 4-celled promycelia.

With the results of all the germination tests reported above in mind, it is possible to make three general statements covering this phase of the question.

(1) Spermogonia are always found in considerable numbers on leaves of Rubus infected with the long-cycled orange-rust *Gymnoconia interstitialis*.

(2) If no spermogonia are present on a leaf showing aecidia of orange-rust the spores will be found to be uninucleated, and on germination they will form 2-celled promycelia.

(3) In strains of the short-cycled rust whose spores develop 4-celled promycelia, spermogonia always precede or accompany the formation of aecidia.

TWO-CELLED PROMYCELIA FROM A STRAIN HAVING SPERMOGONIA

During the prosecution of this survey work there has been found very rarely a form of orange-rust in which spermogonia appear to be fairly well developed, yet the æcidiospores are uninucleated and develop 2-celled promycelia. A Crandall blackberry was found infected with this type. Some of the Eldorado blackberries in Doctor Rankin's experimental plots at Geneva, N. Y., show

spermogonia. A few 2-celled promycelia were seen in cultures from this collection. A blackberry from Doctor White's plot at the Arlington Experiment Farm in Virginia regularly shows rust with spermogonia, yet practically every spore produces a 2-celled promycelium. Sections show that the spores are mostly uninucleated. One other specimen sent in appears to be of the same nature. So far as has been learned, every specimen of this interesting form occurred on a cultivated variety of blackberry. It is the only form with spermogonia so far studied that has uninucleated æcidiospores.

CHARACTERISTIC FEATURES OF PROMYCELIA OF THE SHORT-CYCLED ORANGE-RUST

In the preceding discussion, in order to avoid confusion, no mention has been made of the great variability which may sometimes occur in the form and size of the promycelia which develop when spores from a single æcidium are germinated. Likewise, the promycelia were said to be 2-celled or 4-celled, as the case might be, disregarding the fact that in certain germination tests promycelia with from 3 to 8 cells were found. Furthermore, the number of cells which a promycelium develops does not necessarily correspond to the number of nuclei visible or to the number of sporidia that finally mature. Various types of promycelia, as they appeared in the germination test cultures, will be described. The method of origin of the chains of æcidiospores and the behavior of the nuclei during germination and formation of sporidia will then be considered.

As a rule, the æcidiospores of the strain which seems to omit the spermogonial stage are smaller than those of the long-cycled form. The color characters of the æcidia are not always dependable, but, in general, when the aecidia are rather light yellowish-orange as contrasted with reddish-orange colored (Pl. 1, B, D) the spores will be found smaller, and less uniform in shape and size. These small spores are the ones that regularly produce 2-celled promycelia on germination. The more common types of this promycelium are shown in Plate 5, A to I.[3] Two septa are usually plainly visible, cutting off two cells, leaving the spore and a short piece of the germ-tube without cytoplasmic contents. The promycelia are rather slender and their sporidia are not large. As compared with these structures developing from the large spores matured by other short-cycled strains, they are certainly smaller. In cultures of germinating spores from plant No. 497 no promycelia were found with four sporidia attached. Plate 5, H, shows three sporidia on a 2-celled promycelium which is rather "abnormal". Sometimes by following this type of growth a little further, one will find that the contents of one of the sporidia will disappear so that only two finally are discharged. The sterigmata are frequently long and filamentous (Pl. 5, D). This seems to be due to the conditions under which the spores germinate. Normal sporidia are finally developed.

The more common types (4-celled) of promycelia developed from spores of strains producing spermogonia are shown in Plate 5, J to M. These are 4-celled and will all develop four sporidia.

Certain odd types are also figured. They are, however, perfectly normal. A 9-celled promycelium is shown in Plate 5, N. Some of the cells are dead or empty but six sporidia are being matured. Forked promycelia are not very rare. Figure R shows one with eight cells, six of them alive; one sporidium has been discharged, two more are matured, and one is just forming.

The influence that the conditions attendant on spore germination have on the form of the promycelia is occasionally very astonishing. For example, the spores from plant No. 569 develop in the warm laboratory very long germ

[3] The 4-mm. dry lens was used in drawing the figures in Plate 5; the 1.5-mm. oil immersion lens for Plates 2 to 4, inclusive.

tubes (Pl. 5, V), resembling those of the long-cycled Gymnoconia. They become 4-celled, however, and mature four sporidia. Plate 5, S, shows such a promycelium as it would appear a little later. Five-celled promycelia (Pl. 5, T) with one sporidium from each, are sometimes seen in cultures. The formation of five cells and five nuclei after a reduction division is not the regular order of procedure as commonly understood. These odd types of germination are referred to in order to focus attention on the question of nuclear behavior during and after aecidiospore formation, and to raise the question as to whether or not nuclear fusions followed by reduction divisions are always to be found in the short-cycled orange-rusts.

While every specimen of the rust so far discovered which omits its spermogonial stage is characterized by 2-celled promycelia, as previously noted, there are certainly clear-cut cases where spores from leaves with spermogonia are uninucleated and develop 2-celled promycelia with only two sporidia (Pl. 5, W).

ORIGIN OF AECIDIA WITH BINUCLEATED AECIDIOSPORES

In the light of what is known of the vagaries of Rubus orange-rusts, it is impossible to say what was the nature of the material which Olive (*13*) used as the basis for his cytological work on the orange-rust. It was gathered in Indiana on Rubus sp. He figures the Christman cell fusions referred to as "sexual fusions." The rust that Olive studied evidently conformed in every way to what might be expected of the long-cycled form Gymnoconia.

Kunkel (*5*) presumably did not find anything in his studies of the development of the aecidial sorus of the short-cycled form of the orange-rust to suggest that there might be at times some departure from the regular order of procedure in the growth of the aecidium. According to him the two nuclei originally found in the aecidiospore fuse before germination of the spore and as the germ tube begins to push out the fusion nucleus divides, sometimes while yet in the spore; the first division may also occur in the promycelium. After the second division the cross walls are laid down so that four uninucleated cells comprise the mature promycelium. Each of the four cells then develops a sporidium containing a single nucleus. In the manner of their origin, in the behavior of their nuclei on germination, and in the type of promycelia formed from them, the aecidiospores of *Caeoma nitens* were thus, according to Kunkel, not unlike those of *Endophyllum sempervivi* as reported by Hoffman (*4*) and others.

The writer's studies have been confined to preparations made from material obtained from plants infected with rust whose spores have been germinated.

There are many interesting features in the development of aecidia which need further attention other than the so-called "sexual fusions," but as the question relating to the ultimate form of the promycelium is in the main determined at the origin of the aecidiospore, it will be necessary to give this phase of the subject some attention. Disregarding what occurs when the aecidia of the long-cycled orange-rust are formed (Pl. 2, T), the origin of the chains of spores in those strains where 4-celled promycelia are regularly developed from aecidiospores will first be referred to. Cell fusions (Pl. 2, M to S) in numbers can be found in sections of aecidial primordia of the rust on an Iceberg blackberry No. 543. Spermogonia were developed in large numbers on this plant and germination tests showed that most of the spores germinated as short-cycled, forming quite regularly 4-celled promycelia. Binucleated spores and intercalary cells are cut off in regular order above the fusion cells.

In no case is there any indication in these sections of a fusion of the two nuclei in a spore. Hoffman (*4*) shows that in *Endophyllum sempervivi* the two nuclei

fuse and then often undergo the first division before the spore is shed. The writer's sections of telia of the long-cycled form Gymnoconia show that the two nuclei fuse as the teleutospore reaches maturity. Is it possible that the two nuclei of the short-cycled orange-rust spore never fuse? If so, the reduction division would be omitted, only that standing in place of the homoeotypic division persisting to give the four nuclei of the "normal" promycelium.

In some preparations from an infected loganberry "two-legged" cells are very difficult to find (Pl. 2, L). Very likely such figures represent sections at right angles to the pairing cells so that only one of the "leg" cells appears in the section. It is not impossible that chains of binucleated spores may sometimes, or even regularly, arise by mere nuclear division as claimed by Kursanov (*8*). It is certainly strange that fusion cells are so hard to find in this strain on loganberry.

Rust from a blackberry also shows aecidial chains of 2-nucleated spores (Pl. 2, I, J) where it is also difficult to understand why more examples of two-legged fusion cells are not to be found. The cytology of this form of the orange-rust is being given further attention.

ORIGIN OF SORI WITH CHAINS OF UNINUCLEATED AECIDIOSPORES

There being no question as to the existence of cell fusions, at least in the aecidium primordium of the Gymnoconia orange-rust, the form of rust on blackberry which omits the spermogonial stage will next be considered.

Plate 2, A, represents merely in outline and position the structures in an aecidial sorus of the rust on Crandall blackberry which was obtained May 17, 1922. The uninucleated basal cells arise from uninucleated cells of the sorus primordium. Intercalary cells are cut off and the spores are matured in a normal fashion. There were no cell fusions in this sorus. At B a chain of 2-nucleated spores and interstitial cells is shown adjacent to chains of uninucleated spores. Kursanov (*6*) says, in effect, that in *Aecidium leucospermum* this would mean that the nucleus of the original basal cell simply divided and thereafter 2-nucleated cells are cut off. In Plate 2, D and F, are shown chains of spores in an aecidium from plant No. 497, most of whose spores produce 2-celled promycelia. At F the two lower cells in the chain are clearly uninucleated while the spores above are binucleated, but at E the basal cell is binucleated. In some forms scarcely a binucleated spore can be found in a sorus. At G is shown a very young sorus, and at H, a somewhat older one where from first to last the cells are uninucleated.

As noted, cell fusions regularly occur in the aecidium of the long-cycled orange-rust (Pl. 2, T). By triple or quadruple fusions, 3 and 4 nucleated spores presumably may arise as in the other rusts. What would this mean in germination and in infection? In forms of the short-cycled Caeoma rust with spermogonia where cell fusion would be expected to occur frequently, binucleated spores may possibly arise without such fusions. Then, too, 3-nucleated spores are not at all rare in these forms (Pl. 2, K). In strains without spermogonia and with uninucleated spores originating without fusion there are also 2-nucleated spores, possibly formed as the result of nuclear division and also from cell fusions. By studying stained preparations of germinating spores the writer becomes more thoroughly convinced that the orange-rusts represent forms which are not fixed in their life cycles. Bearing in mind the facts regarding the methods of origin of the aecidiospores one may understand more readily why there is such a great variation in the forms of promycelia.

NUCLEI IN PROMYCELIA

In Plates 3 and 4 are shown promycelia in various stages of growth from the germination of spores on to the formation of sporidia. The preparations studied were fixed and stained. The shaded areas merely represent living substance, cytoplasm and nuclei. The magnification is the same as used in Plate 2, which is somewhat higher than used in Plate 5, with which the figures should be compared. In Plate 3, A to K, are represented promycelia from spores of the rust on plant No. 497. At A and B division of the original nucleus has taken place; at C, the original nucleus has moved out into the promycelium where it will divide only once. The first cross wall is sometimes laid down before the first division (D), but most frequently division occurs in the spore itself. Any departure from standardized behavior on the part of the nuclei is reflected at once in the promycelium (J, K). In one case the promycelium has only two cells, but one of them has two nuclei. In the one shown at K there are three cells, with one, two, and three nuclei, respectively.

The other promycelia (Pl. 3, L to R), are from spores in aecidia of strains which produce spermogonia. It should be noted that spores which develop promycelia with three or more cells are correspondingly larger. At M is shown a promycelium with six cells, but only four of them are alive and nucleated, while at Q there is shown a 4-celled promycelium, one cell of which is dead, the other three contain two nuclei each. At N is shown a sporidium with three nuclei; one cell of the promycelium was dead. Perhaps its nucleus migrated into the upper cell and entered its sporidium. A binucleated sporidium arises as the result of division of its original nucleus, generally after the sporidium is matured.

Further details in the growth of promycelia are shown in Plate 4, A to J, from plant No. 489, where most of the aecidiospores are uninucleated. At A, the germ tube is put out before nuclear division, while nuclear division has already occurred in the spore shown at B. At C, the two daughter nuclei are passing out of the spore. Nuclear division is shown taking place in the promycelium at D. In cultures of this rust from plant No. 489 a great majority of the spores germinate in the forms shown from A to K. Figures L to S show types of germination from spores which had originally two or more nuclei. Three nuclei were clearly visible in the large spore shown at L. One nucleus in each of the cells of promycelia shown at M, Q, and O is degenerating. At N, two nuclei are entering the sterigma. The central cell of the 3-celled promycelium at S has two nuclei, the others, one each. Figures T to V in Plate 4 show the prevalent type of promycelium from the rust on plant No. 493. At X and Y are shown 4-celled promycelia, but it seems two cells in each case have captured the nuclei, three and three at X, two and four at Y. If the terms "normal" and "abnormal" must be used in describing promycelia, it should be done for convenience only, meaning by "normal" the most common or prevailing type. All of the odd forms could probably be looked upon as regular if one only knew the whole story. The writer has found aecidiospores with as many as seven nuclei in a young sorus whose spores were most regularly uninucleated.

DISCUSSION

The short-cycled orange-rusts of Rubus have much in common with those included under the genus Endophyllum. According to Hoffman (*4*), whose work has been confirmed by Moreau (*12*), cell fusions occur at the origin of the aecidiospore chains in *E. sempervivi*. The two nuclei resulting from the conjugate divisions of the nuclei contributed by the fusing cells, being cut off in the spore cell, fuse before the spore is shed. The fusion nucleus, which is much larger than either of the original nuclei, then undergoes a division so that the mature spore

again contains two. This first division may be delayed and later occur in the germ tube. The first septum is laid down before the second division occurs. Finally, a single uninucleated sporidium develops from each of the four cells. Such a course of procedure, following as it does what is known of nuclear behavior during the formation and germination of the teleutospore of the rusts, has been considered by some authorities to be sufficient evidence to warrant calling the aecidiospore of an Endophyllum a teleutospore. Evidence is accumulating very rapidly, however, to indicate that while the spore forms of the rusts as morphological entities are fixed because of their phylogenetic antecedents, the methods by which a particular spore form arises and the nuclear behavior at its origin and during germination are far from generalized, orderly, or fixed processes.

In the Florideae the methods of origin of the cystocarps and nuclear behavior in the cell activities involved in the formation of carpospores vary in some essential details with almost every genus in the five orders. One certainly must expect that their rust offspring will be just as individualistic.

There is an Endophyllum, *E. euphorbiae-silvaticae*, in which, as proved by Sappin-Trouffy (*14*) and Moreau (*12*), the two nuclei originally entering the aecidiospore never fuse. They pass out into the young promycelium and divide once, giving the four nuclei for the maturing of four sporidia. According to Maire (*10*), one of the two original nuclei of the spore of *E. valerianae-tuberosae* degenerates so that a reduction division could not follow.[4] Moreau (*11*, *12*) has furthermore given convincing proof that there is a form of *E. euphorbiae* with uninucleated aecidiospores and that there are no cell fusions in its aecidium. Her figures of the germinating spores and of the promycelia are, however, not all that could be desired. Whether or not Moreau believes that the promycelia from these uninucleated spores would "normally" be 4-celled and produce four spores is not clear from her papers. Nothing in her figures suggests such a thing.

Certainly one finds no explanation of the peculiarities of the orange-rust in *Puccinia malvacearum*, the only rust lacking spermogonia that has been thoroughly studied as to its cytology and physiology. It is a perfectly "normal" short-cycled form, except for its lack of spermogonia. In the absence of any definite knowledge respecting spore formation and the nuclear phenomena in those other rusts which lack spermogonia it would be futile to speculate on the meaning of the loss of these structures in the strain of *Caeoma nitens* under discussion.

Knowing nothing as to the development of spermogonia by the "*uninucleatum*" strain of Endophyllum, and nothing regarding the number of nuclei and cells in the promycelium except that the germ tubes that were obtained did have septa, it can only be said that further research may show that this Endophyllum may resemble the orange-rust not only as to the origin of its uninucleated spores, but also in the suppression of its spermogonia as well as in the formation of 2-celled promycelia. Germination of the spores of the form of *E. euphorbiae*, which Maire (*10*) claims becomes uninucleated by degeneration, should also be studied.

Two other European cytologists are accumulating evidence proving that life histories of species of rusts are not always as fixed events as has heretofore been generally believed. Kursanov (*7*) has pointed out that in certain sori of *Aecidium punctatum* the peridial cells and their interstitial cells are uninucleated. The spores in such a sorus are uninucleated except that here and there a spore has two nuclei clearly as the result of nuclear division. Nothing is said of the manner of germination of the uninucleated spores. There is a form of *Aecidium*

[4] In a later paper (MAIRE, R. L'ÉVOLUTION NUCLÉAIRE CHEZ LES ENDOPHYLLUM. Jour. Bot. [Paris] 14:80-97, 369-382, pl. 3. 1900) he figures 2-celled promycelia, failing to furnish any evidence by figures, that the aecidiospore becomes uninucleated by degeneration.

leucospermum which occurs frequently in the vicinity of Moscow and which Kursanov (*6*) finds has sori with chains of uninucleated aecidiospores. No cell fusions take place at their origin. The spores germinate like the ordinary aecidiospore, except that the germ tube has one nucleus instead of two. The spores with two nuclei are much larger than those with only one, whereas Moreau (*12*, p. *182*) found that the uninucleated spores of her Endophyllum corresponded in size with those described for the species. Occasionally a spore in one of the chains is binucleated. Such a case is clearly the result of a nuclear division. Where a chain of binucleated spores is found in a sorus the possibility of a cell fusion at the base is not precluded.

Lindfors (*9*) has more recently given a most interesting series of brief discussions of life histories of certain rusts. He has signally departed occasionally from the practice of trying to make what he finds in his material conform to the commonly accepted notions of cell fusions and to the doctrines relating to the fixity in rust cycles. For example, he finds that in *Uromyces acetosae* the first telia develop close to the aecidia. He figures an aecidium and a telium arising out of a common uninucleated mycelium. He also claims that the binucleated condition is not limited as to its origin, to cell fusions at the base of a sorus. Although cell fusions occur in *Puccinia arenariae*, without spermogonia, the promycelium has only two cells. Two binucleated sporidia are formed.

Up to the present time one of the greatest obstacles to the understanding of the workings of evolution in the Uredineae is our proneness to believe in the fixity of what are called genera and species. Owing to the immediate need for some systematic treatment of rust parasites, which were being discovered attacking our economic plants, much of the preliminary work, of necessity, has been based on dried material. Furthermore the cytological work has mostly been confined to a study of the so-called sexual fusions. The desirability of gathering, for fixation, material obtained as the result of extended culture work was clearly not of prime necessity in those days when Blackman and Christman were making their discoveries relating to nuclear migrations and cell fusions occurring at the origin of the aecidium. Moreau and Kursanov did not supplement their cytological work on uninucleated aecidiospores with culture work, and nothing was said regarding the presence or absence of spermogonia in those forms. Judging from Moreau's figures of the promycelia, which do not show any nuclei, it would not be surprising to find that if the uninucleated spores of that Endophyllum were germinated under conditions sufficiently normal to lead to the formation of sporidia, the promycelium would be found to be regularly 2-celled with one nucleus in each cell.

Blackman (*1*) saw in the sterile cell above one of the fusing cells the ancestral red alga trichogyne, and he saw in the migrating nucleus passing up into the basal cell (the "egg cell") a substitute for the now functionless spermatium. This view has long been discarded. In all of the more or less deep-seated aecidial primordia the fusing cells are in reality merely intercalary cells of the stroma-like, space-making, and food-storing hyphal plexus. The fusions are of exactly the same nature as are those occurring between the accessory sterile and auxiliary cells in the red algae. The resultant is the accumulation of materials and vigor for the building of numbers of reproductive spore bodies.

Christman's (*2*) suggestion that the cell fusions occurring between two equal cells must represent the sexual fusions of the ancestral Spirogyra-Mucor of the Conjugateae, while not generally accepted in this particular sense, nevertheless has gone far to establish firmly in the literature the terms sexual cells (gametes) and sexual fusions for those purely secondary fusions occurring between cells which have no homologies whatever to sexual organs, spermatia and oogonia, of any possible ancestral form.

There is no structure known in the rusts which takes the place of or is the homologue of an egg apparatus. The factor which stands for such an organ was either discarded when the rusts were evolved, or it became recessive to the extent that, for all that is known, the egg structure is always suppressed. It is this loss or impotency of the factor for that element of femaleness represented by the egg that distinguishes a rust most fundamentally from its ancestral alga.

The existence of a strain of any rust characterized by the development of a 2-celled promycelium has not hitherto been reported. Inasmuch as the suppression of spermogonia has been said in this paper to be so constantly associated with the production of uninucleated spores with abbreviated promycelia, it might appear as though the writer looks upon the production of spermogonia as inseparably connected with the faculty for initiating cell fusions. This may indeed be true to a certain extent, but, as has been noted, the development of spermogonia does not always insure cell fusions, for a few cases of infection have been found where, though spermogonia were present, the spores were uninucleated.

There is, of course, something in the inheritance of the rusts that determines when and where these cell fusions shall take place, as well as the nature of the cells fusing. The five great orders in the Florideae are distinguished mainly on the basis of the form and disposition of the carpogonial branches and auxiliary cells, and on the nature of the cell fusions which follow as secondary events. The gonimoblasts or ooblastema filaments, sporophytic outgrowths from the fertilized egg, in many genera are also involved in these secondary fusions preliminary to the development of carpospores. It is to these secondary cell fusions in which the auxiliary cells of the red alga take the leading part that we must look for the homologues of the rust fusing cells.

There being no organ such as an egg apparatus in the rusts, the spermogonia, though they are so well developed in many species, can not carry out their primary male sexual function. Nevertheless, there may be a series of activities in the growth of the rust bound up ("linked") with or influenced by the very inheritance which manifests itself morphologically in the shape of spermogonia. The fusions between the ooblastema filaments and auxiliary cells, and the other cell fusions in the red algae may very well be determined by the stimulus resulting from the presence of some of that element of maleness normally derived from the spermatium during fertilization. In the absence of the sexual fusions certain auxiliary cell fusions would not occur.

Femaleness in the red algae outwardly expresses itself primarily in the organization of the carpogonial branch bearing the egg cell and its trichogyne. Auxiliary cells are a secondary manifestation or accompanying phenomenon. In a dioecious alga one should not expect to find auxiliary cells borne on the male plant. Femaleness in the orange-rust—that is, the power to develop an egg apparatus—has been lost. Auxiliary cells represented by the cells in the sorus primordium are the secondary expressions. These cells are capable of fusing, usually in pairs, under a certain stimulus.

Maleness in the red algae is expressed in the form of antheridia (spermogonia) producing spermatia. These bodies function primarily in fecundation, and, in certain groups, secondarily in the cell fusions subsequent to fertilization. In the rusts maleness has persisted as shown by the development of spermogonia with their spermatia, which are primarily functionless in the absence of an egg apparatus. This condition is associated with the occurrence of accessory cell fusions, culminating in Gymnoconia in nuclear fusions in the teleutospore.

A long-cycled rust becomes short-cycled without losing the power to develop that morphological structure, the promycelium. A promycelium may be formed without there having taken place a nuclear fusion previously, in which case

obviating the necessity for a reduction nuclear division. For this reason the number of cells in a rust protobasidium need not always be four. The presence of some male element exercising its secondary function indicates maturity and cell fusions will occur, while inhibition of such a development is manifested by the omission of cell fusions.

Aside from the immediate developments dependent directly on fecundation, there is frequently a whole series of activities well known and recognizable in sexual reproduction in plants and animals which result from the expression of sexuality in a secondary capacity. One can conceive of such a thing as the agent inaugurating cell fusions in the short-cycled orange-rust being ordinarily "linked" with or dependent on the factor which stands for spermogonial development, yet occasionally becoming separated from this factor. Ordinarily then the appearance of spermogonia would be followed by cell fusions, except where there has been a transfer of the factors for fusions to some other determinant where it could no longer express itself normally.

The Gymnoconia as noted becomes short-cycled by omitting or suppressing the telial stage. If that combination of factors which was instrumental in bringing about a shortening of the life cycle were unconnected with maleness in any way, the rust would develop its spermogonia, cell and nuclear fusions would follow as before, and the spores would germinate with 4-celled promycelia. If one should be able to prove that in the short-cycled form there are no nuclear fusions and that chains of binucleated or multinucleated spores may arise without cell fusions, it would be further evidence of the complexity of the factors involved in the development of the various spore forms.

The writer has pointed out that the spores with one nucleus are smaller than than those with two or more. This agrees with what Kursanov (*8*) says of the uninucleated spores of *Aecidium leucospermum*. Kunkel (*5*) figures uninucleate aecidiospores of the short-cycled orange-rust, but he interprets them as resulting from nuclear fusions. It should be noted that in his figures the uninucleated spores are in both cases much smaller than the binucleated spores.

The very fact that promycelia with irregular or odd numbers of cells—three to nine are found—and that as many as five nuclei may occur in one cell, would indicate that this multicellular, multinuclear condition has not arisen as a result of fusions of nuclei in pairs in the aecidiospore followed by regular reduction divisions.

It is inconceivable that a 7-nucleated aecidiospore which was found in an aecidium, the other spores of which were mostly uninucleated, could have been the result of the fusion of seven cells. When such a spore germinates, a promycelium is formed which, from the nature of its origin, will be normal; but from a viewport fixed by previous misconceptions, will be abnormal. The writer has found that in certain strains of the short-cycled orange-rust practically every promycelium is 4-celled. This can almost be determined in advance by the form and size of the aecidiospores. In any culture where the spores vary considerably in shape and size some "abnormal" promycelia will be developed.

There is a rather fascinating hypothesis which might be brought forward to account for the frequency with which one finds the omission of the spermogonial stage is associated with the omission of cell fusions and with the production of 1-nucleated spores in the aecidium.

If as Kniep maintains in the anther smut, *Ustilago antherarum*, the two sporidia at one end of the promycelium are "plus" and the other two "minus," then the host can be infected so that the rust develops normally only when a sporidium of each sort takes part in the infection. Infection from a single sporidium might be possible only in case the strain happened to be capable of, say, an androgenetic development assuming that + and − refer to femaleness and maleness. In this

event, spermogonia would be formed, but cell fusions being omitted the aecidiospores would be uninucleated. If the sporidium from the opposite end produced infection it could only be by a gynogenetic process. In such an event, no spermogonia would appear, no cell fusions would occur, and 1-nucleated spores would arise.

The two strains evolved should remain or continue to be true to form, but by bringing about a double infection with a sporidium of each sort one would obtain a strain with spermogonia, cell fusions, 2-nucleated aecidiospores and 4-celled promycelia. The difficulty with which one is able to infect a blackberry systemically and the rareness with which natural systemic infections occur in comparison with the vast number of spores shed, would suggest that this hypothesis should not be entirely ignored. One is reminded that future infection work with the orange-rusts should be carried on with spores at least from a single plant and ultimately with single aecidiospores and even with single sporidia.

SUMMARY

Certain strains of the short-cycled orange-rust on Rubus do not develop spermogonia except perhaps as they are aborted or vestigial.

The aecidiospores in such strains are for the most part uninucleated. Binucleated or multinucleated spores are sometimes present in such aecidia.

Cell fusions do not occur at the origin of a chain of uninucleated aecidiospores.

The number of nuclei in an aecidiospore may be increased by nuclear divisions.

The size of an aecidiospore varies with the number of nuclei present at its maturity. The uninucleated spores are much smaller than those with two or more nuclei.

A spore with one nucleus germinates normally; its nucleus divides once so that a 2-celled promycelium which matures two sporidia is formed.

The number of cells in a promycelium and the number of sporidia matured is not fixed, but depends to a great extent on the number of nuclei in the aecidiospore. A polynucleated spore is very large and would probably give rise to a promycelium with more than four cells and very likely some of the cells would contain more than a single nucleus.

Degeneration of nuclei sometimes occurs in the cells of promycelia and certain of its cells may die, so that the number of sporidia eventually formed does not necessarily correspond to the number of nuclei originally present.

So far as yet observed spermogonia are always matured by the long-cycled rust *Gymnoconia interstitialis* and cell fusions occur.

Strains of the rust which commonly form 4-celled promycelia when the aecidiospores are germinated always develop spermogonia. In this form the binucleated aecidiospores arise as the result of cell fusions.

That nuclear fusions always occur in the aecidiospore or its promycelium in the short-cycled orange-rust is questioned.

So-called "abnormal" promycelia with other than the traditional number, four, cells are accounted for by nuclear behavior during spore development and germination. When a spore has but one nucleus, that nucleus divides only once during the formation of the 2-celled promycelium.

Binucleated spores arising from cell fusions usually develop 4-celled promycelia, possibly without nuclear fusions followed by reduction divisions. On this principle a precocious division of the nuclei in a spore followed by a multicellular promycelium need not introduce a new element into the inheritance of the rust.

LITERATURE CITED

(1) Blackman, V. H.
1904. on the fertilization, alternation of generations and general cytology of the uredineae. Ann. Bot. 18: 323–373, illus.

(2) Christman, A. H.
1905. sexual reproduction in the rusts. Bot. Gaz. 39: 267–275, illus.

(3) Dodge, B. O.
1923. effect of the orange rusts of rubus on the development and distribution of stomata. Jour. Agr. Research 25: 495–500, illus.

(4) Hoffman, A. W. H.
1912. zur entwicklungsgeschichte von endophyllum sempervivi. Centbl. Bakt. (II) 32: 137–158, illus.

(5) Kunkel, L. O.
1914. nuclear behavior in the promycelia of caeoma nitens burrill and puccinia peckiana howe. Amer. Jour. Bot. 1: 37–46 illus.

(6) Kursanov, L.
1913. sur la morphologie des urédinées. Trav. Sec. Mycol. et Phytopathol. Soc. Bot. Russ. 1: 19–21, illus. [In Russian. French resumé, p. 19–21.]

(7) ———
1914. über die peridienentwicklung im aecidium. Ber. Deut. Bot. Gesell. 32: 317–327, illus.

(8) ———
1922. recherches morphologiques et cytologiques sur les urédinées. Bul. Soc. Nat. Moscou (n. s.) 31: 1–129, illus.

(9) Lindfors, T.
1924. studien über den entwicklungsverlauf bei einigen rostpilzen aus zytologischen und anatomischen gesichtspunkten. Svensk Bot. Tidskr. 18: 1–84, illus.

(10) Maire, R.
1900. quelques urédinées et ustilaginées nouvelles ou peu connues. Bul. Soc. Mycol. France 16: 65–72.

(11) Moreau, Mme. F.
1911. sur l'existence d'une forme écidienne uninucléée. Bul. Soc. Mycol. France 27: 489–493, illus.

(12) ———
1914. les phénomènes de la sexualité chez les urédinées. Botaniste 3: 145–284, illus.

(13) Olive, E. W.
1908. sexual cell fusions and vegetative nuclear divisions in the rusts. Ann. Bot. 22: 333–360.

(14) Sappin-Trouffy, P.
1896. recherches histologiques sur la famille des urédinées. Botaniste 5: 59–244.

(15) Werth, E., and Ludwigs, K.
1912. zur sporenbildung bei rost- und brandpilzen. Ber. Deut. Bot. Gesell. 30: 522–528, illus.

PLATE 1

A to E.—Types of orange-rust on Rubus

A.—Short-cycled, spermogonia and four-celled promycelia. Waxy spores heaped up in the sori.

B.—Short-cycled, no spermogonia, 2-celled promycelia. Yellowish spores waxy.

C.—Three yellowish short-cycled sori like those in B, other sori long-cycled.

D.—All sori long-cycled.

E.—Light orange empty sori, long-cycled; reddish orange sori with waxy spores are short-cycled. Two long sori partly short-cycled and partly long-cycled x 12.

B. C. and D from the same plant.

A

B

C

D

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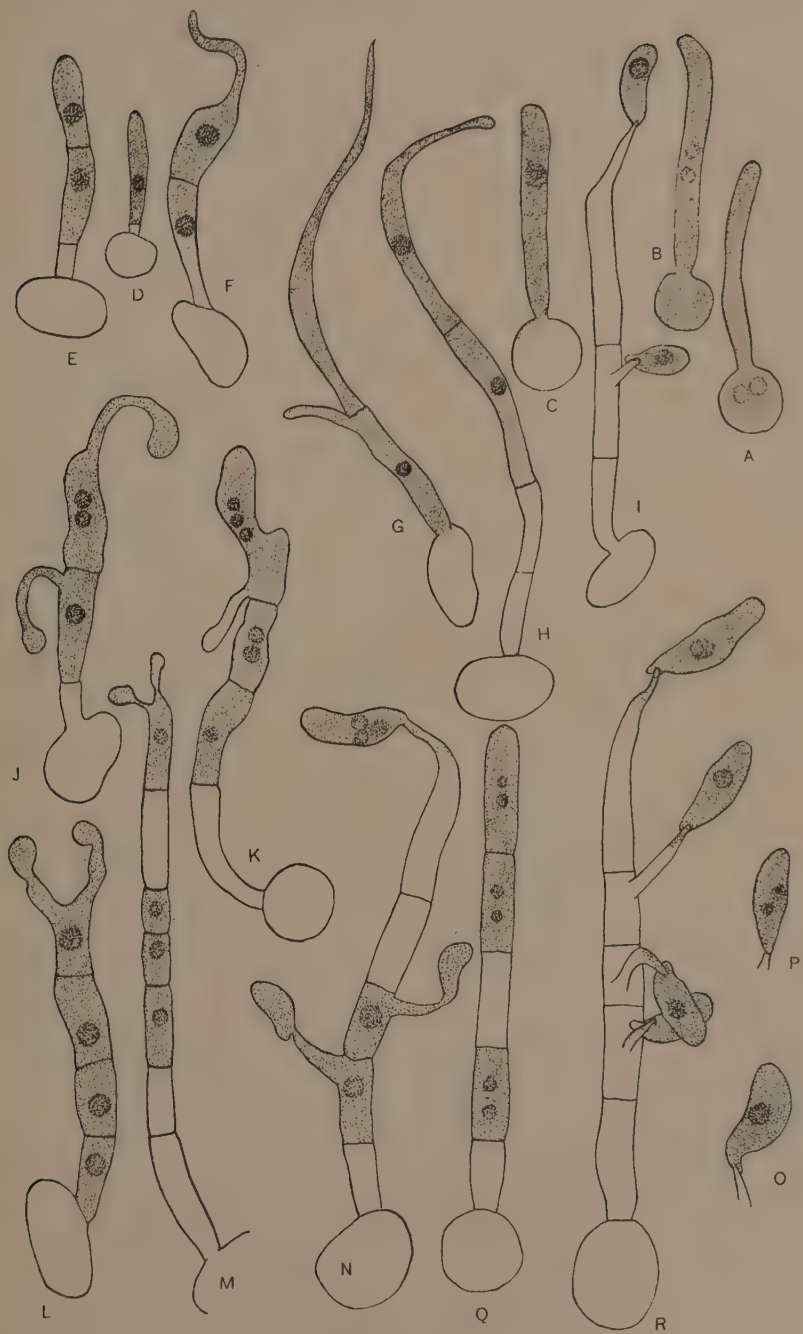
PLATE 2

A to C.—From sections of a young aecidium on Crandall blackberry. No Spermogonia, no cell fusions, mostly 1-nucleated spores. D to F.—Spore-chains from aecidium on plant No. 497. G.—A young primordium. H.—Older sorus; all cells 1-nucleated. I to S.—From strains maturing spermogonia. T.—Spore-chain from the long-cycled Gymnoconia on *Rubus occidentalis*

PLATE 3

A to J.—From the short-cycled rust on plant No. 497 (cf. Pl. 5, A to I). K to R.—From strains producing spermogonia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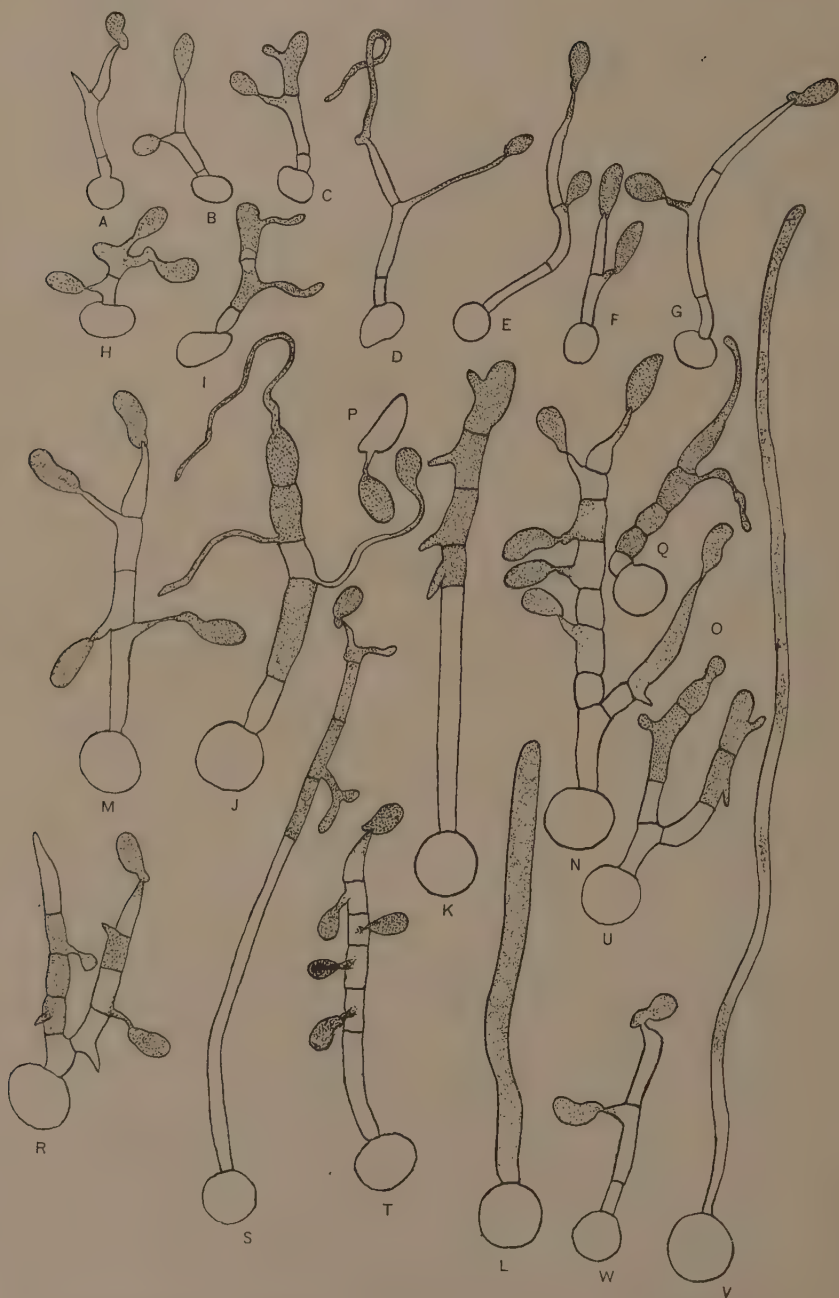
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PLATE 4

A to Q.—From short-cycled rust on plant No. 489. R to Z.—From a strain of the short-cycled rust on plant No. 493

PLATE 5

A to I.—2-celled promycelia from germination tests from plant No. 497. Small spores slender promycelia. J to V.—Promycelia from strains which develop spermogonia. Cells without cytoplasmic contents not shaded. W.—2-celled promycelium from a strain having spermogonia and 1-nucleated spored.

Uninucleated Aecidiospores in Caeoma nitens

PLATE 4